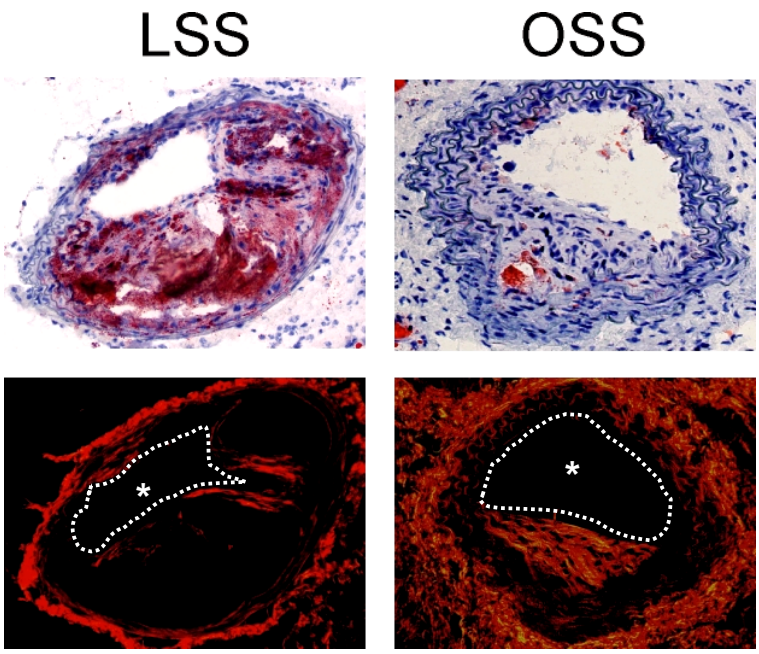
Supplemental data

Supplemental Table 1: Expression levels of chemokines in carotid arteries at different time points after cast placement in mice on Western diet. Shown are the expression levels of RANTES, eotaxin, ELC, NAP-2, SDF-α, and CCL14 after one, three, and nine weeks of cast placement. Data was obtained by QPCR using pooled amplified RNA samples. (10 animals per pool, N=3). *P<0.05 versus control. #P<0.05 versus low shear stress.

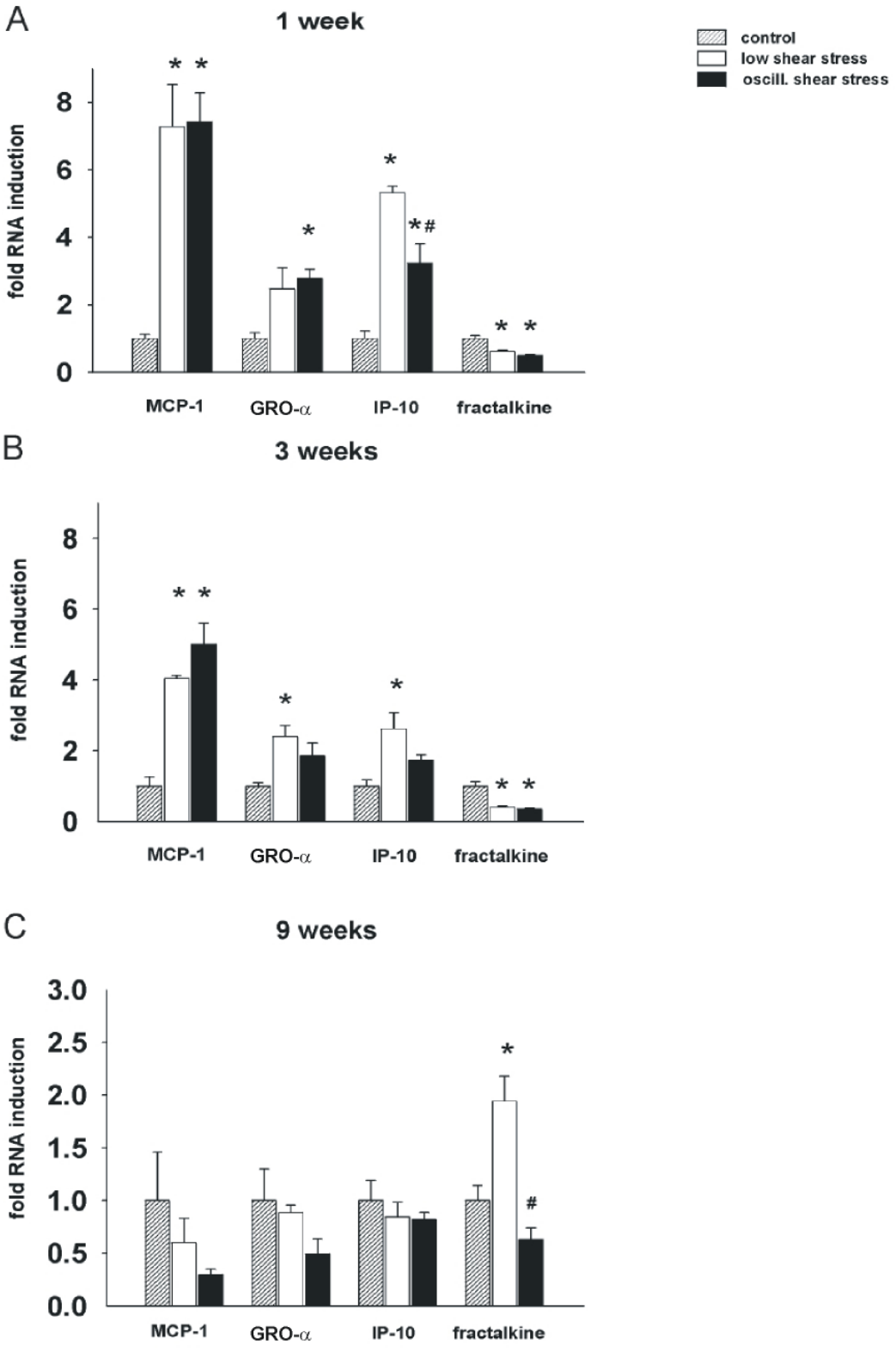
	control			low shear stress			oscillatory shear stress		
chemokine	1 week	3 weeks	9 weeks	1 week	3 weeks	9 weeks	1 week	3 weeks	9 weeks
RANTES	1.00 ±0.10	1.00 ±0.18	1.00 ±0.69	0.68 ±0.12	0.15 ±0.06*	0.37 ±0.14	0.72 ±0.14	0.29 ±0.28*	0.32 ±0.06
eotaxin	1.00 ±0.15	1.00 ±0.05	1.00 ±0.30	0.39 ±0.09*	0.15 ±0.07*	0.31 ±0.03	0.23 ±0.01*#	0.44 ±0.16*	0.48 ±0.28
ELC	1.00 ±0.07	1.00 ±0.33	1.00 ±0.13	0.60 ±0.07*	0.47 ±0.03	0.41 ±0.16*	0.42 ±0.13*	0.33 ±0.01*#	0.12 ±0.03*
NAP-2	1.00 ±0.14	1.00 ±0.34	1.00 ±0.12	1.00 ±0.04	1.08 ±0.12	0.85 ±0.07	1.14 ±0.17	1.41 ±0.00	0.39 ±0.12 *#
SDF-α	1.00 ±0.09	1.00 ±0.15	1.00 ±0.24	0.91 ±0.11*	1.34 ±0.07	0.32 ±0.20	0.41 ±0.32#	1.82 ±0.34	0.34 ±0.25
CCL14	1.00 ±0.17	1.00 ±0.50	1.00 ±0.56	0.36 ±0.09*	0.34 ±0.22	0.48 ±0.24	0.49 ±0.05*	0.50 ±0.28	0.54 ±0.30

Supplemental Table 2: Sequences of primers used for gene expression analysis.

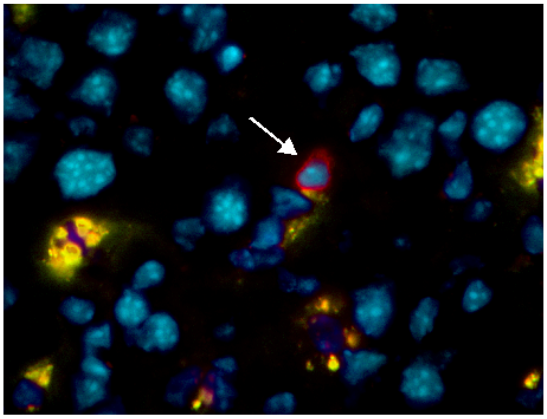
Gene	5'primer	3'primer
MCP-1	5-AATCCAGATTCAACACTTTCAATG-3	5-AAACAATACCTTGGAATCTCAAACA-3
GRO-α/KC	5-CATGTAGAAAGCCCATCTGGA-3	5-CTGCAATCAGAAAAGAGTCATTG-3
RANTES	5-GCACAGCAAACCCAAGAAAT-3	5-ACTTGTGGGCAAGAGGGTTT-3
Eotaxin-1	5-GACCTGTAACTCACTGTGTAGACCA-3	5-ATTCTGGCTTGGCATGGTAG-3
ELC	5-CCAGCCAAGTCTGTGCCTA-3	5-CGGCTTTATTGGAAGCTCTG-3
SDF-α	5-CAGCCACGGTGTATTTTTCC-3	5-CAGCATGAAACAATTAGCATTTT-3
NAP-2	5-TCATGACCATGTTTTTCCAA-3	5-TGATTTTATTGTGTTCAGTTTCAAG-3
IP-10	5-CACATGACCATTTCATGTCAGTT-3	5-AAAACCGTCCAATACCTTTTGT-3
fractalkine	5-TCGGACTTTGTTGGTTCCTC-3	5-CAGACATTGGTAATGATGCTTGA-3
CCL-14	5-GAAGGCACAGCTCAGAGAGA-3	5-AAGCAGGGAAGCTCCAAGAG-3
HPRT	5-CAGCCAACACTGCTGAAACA-3	5-TCAGGAGAGAAAGATGTGATTGA-3



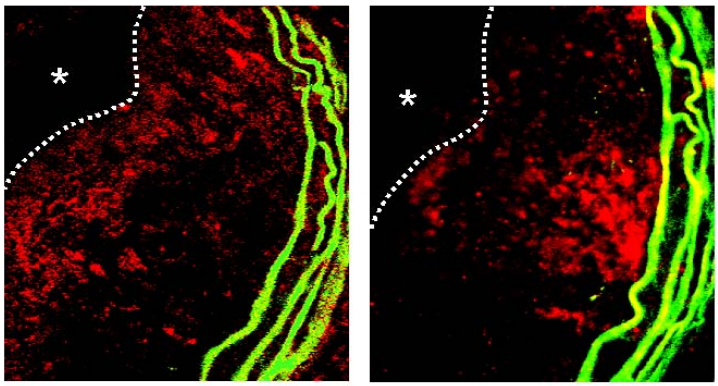
Supplemental figure 1: *Representative cross-sections of carotid arteries 9 weeks after cast placement in ApoE-/- mice on a Western diet stained for lipids using Oil red-O (upper panels) or collagen using picrosirius red (lower panels). Asterisks indicate the position of the lumen, while the white dotted lines demarcate the boundaries between lumen and intima. Low shear stress: LSS; oscillatory shear stress: OSS.*


A
1 week
control
low shear stress
oscill. shear stress
fold RNA induction
0
2
4
6
8
MCP-1
GRO-α
IP-10
fractalkine
B
3 weeks
C
9 weeks
0.0
0.5
1.0
1.5
2.0
2.5
3.0

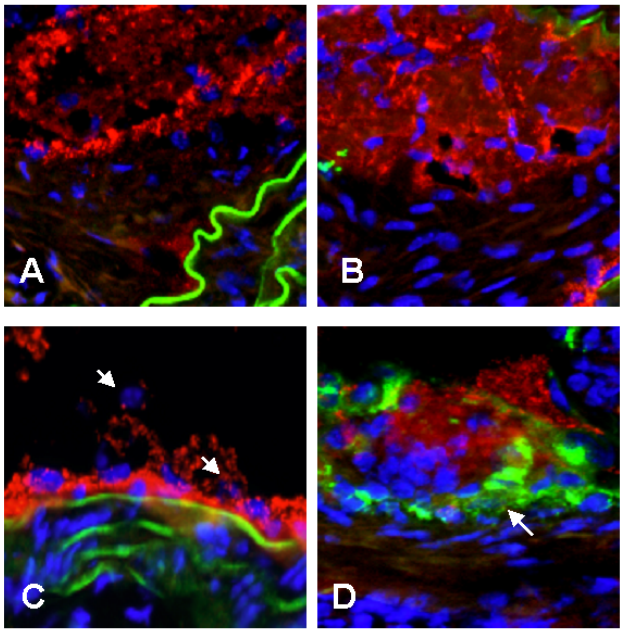
Supplemental figure 2: *Expression levels of upregulated chemokines in carotid arteries at different time points after cast placement in mice on a Western diet. Expression profiles were measured by QPCR using amplified RNA samples (10 animals per pool, N=3). The expression levels of MCP-1, GRO-α/KC, IP-10, and fractalkine have been measured after (A) one week, (B) three weeks, (C) and nine weeks of cast placement. *P<0.05 versus control. #P<0.05 versus low shear stress.*



Supplemental figure 3: *Cross-section of the carotid artery 9 weeks after cast placement in ApoE-/- mice on a Western diet stained for Ly49G2 (red signal) and cell nucleus (blue signal) in the intimal area. Yellow Signal is auto-fluorescence of apoptotic cells.*



Supplemental figure 4: *Serial cross-sections of an atherosclerotic lesion in ApoE-/- mice on a Western diet stained for CX3CR1 (red signal left) and fractalkine (red signal right) in the intimal area. Also visible is the green auto-fluorescence of the elastic lamina. Asterisks indicate the position of the lumen, while the white dotted lines demarcate the boundaries between lumen and intima.*



Supplemental figure 5: *Cross-sections of atherosclerotic lesions in ApoE-/- mice on a Western diet stained for P-selectin (red signal) (A, B and C) and Willebrandt factor (green signal) (D) in the intimal area. Cell nuclei are stained blue with DAPI. Also visible is the green auto-fluorescence of the elastic lamina (A, B, and C). Platelets-leukocytes conjugates and platelets rich thrombi are detected lining the endothelium on top of the atherosclerotic plaques (A and B). Platelet decorated leucocytes were also detected (indicated by white arrows) on atherosclerosis-free activated endothelium in the low and oscillatory shear stress regions (C). As P-selectin is also expressed by endothelial cells, we double stained with the more endothelial cell specific von Willebrandt factor (green signal) to segregate the endothelium from the platelets conjugates. This identified the P-selectin positive morphological structures as platelets-leukocytes conjugates covering the von Willebrandt factor positive endothelium (indicated by a white arrow) (D).*